Differentiating Between Genetic Engineering and DNA-Informed Breeding in the RosBREED Project

Cameron Peace, DNA-Informed Breeding Team Leader, Michael Coe, Project Evaluation, Drew Wilson, Extension Team, and Mercy Olmstead, Extension Team Leader

The RosBREED project uses “DNA-informed breeding” rather than “genetic engineering” to genetically improve horticultural quality and disease resistance. What’s the difference?

**Monitoring or modifying**

DNA-informed breeding, also known as marker-assisted breeding, uses direct monitoring of the genetic code (DNA) of many individuals by DNA profiling, to inform decisions in the selective breeding process. Genetic engineering uses direct modification of the DNA of an individual that itself has previously been selectively bred, creating an individual almost the same as the original but with one or a few new genes added to provide a particular attribute not possible or not readily achievable by normal crossing of parents.

**Precise allele additions or wholesale reshuffling**

In everyday selective breeding, alleles (the variant forms of genes) of the parents get reshuffled, and multiple unique offspring are created. The two parents combined in each cross contribute a random half of their own alleles from across their genomes, many of which may engender undesirable attributes. The two parents used in selective breeding are usually from the same species, but might be from closely related species. The pool of species with which a crop is able to naturally hybridize can offer a vast array of valuable alleles. For example, most Prunus species can hybridize with each other, allowing sharing of alleles among the crops of peach, almond, plum, apricot, cherry, and hundreds of other Prunus species. Alleles from a wild parent of the same or another species can be helpful (e.g., providing hardiness or disease resistance) but might be accompanied by alleles that are not desirable (e.g., for small, astringent fruit). Despite the imprecision, sometimes breeders serendipitously discover delightful and valuable attributes in offspring that were not expected. The ultra-crispness of ‘Honeycrisp’ apple is a good example, although breeders now using that cultivar and its descendants are purposely aiming to incorporate that ultra-crisp attribute.

By enhancing the crossing process with DNA information, desired combinations of alleles can be targeted with better accuracy – and even improved creativity. Parents with a preponderance of alleles of interest and that are more likely to pass them on to offspring (because they have two copies rather than one) are used. Beyond consideration of individual parents, pairs of parents can be chosen that together are likely to provide a larger proportion of promising offspring (that are less likely to be discarded later) or that provide a rare allelic combination that might lead to a breakthrough attribute. Pairs of parents that carry desirable alleles for a trait but from differing ancestral sources are more likely to lead to extremely desirable levels of the trait when such alleles are combined in offspring. And crossing parents genetically distinct from each other for larger portions of their genomes infuses diversity into the resulting family that can enhance serendipity.
Featured Team: Data Management Team

Dorrie Main, Data Management Team Leader, WSU and Desmond Layne, Extension Team, WSU

We are in the age of “big data” – massive amounts of information are available on our Dropbox accounts, on supercomputers, and in server farms housed in large buildings to run the internet. In the RosBREED project, all of the phenotyping and genotyping that our scientists do must be quality-checked, stored and, importantly, mined for information that helps us to make progress toward our objectives.

Housing and making all this data accessible to the Rosaceae community is a daunting task, ably handled by Dorrie Main at Washington State University. Dorrie also leads the Genome Database for Rosaceae (GDR), the backbone of the international Rosaceae genomics, genetics, and breeding community’s system for storing genomic and genetic data and making it available to scientists. Many of the Data Management Team are also involved in the GDR, making for a seamless exchange and distribution of data (Figure 1).

The main activities of the Data Management Team are to: 1) make maps of and upload trait information generated by RosBREED scientists; 2) develop a web interface to help RosBREED participants access this data; 3) conduct various bioinformatic analyses; and 4) develop RosMAP, a genome-searching tool that identifies common regions influencing traits in multiple crops, taking advantage of the similarity in genomes among the Rosaceae family. Dorrie and her team are also leading the evaluation of a mobile phenotyping application called FieldBook. Developed by the wheat breeding program at Kansas State University (Rife and Poland, 2014), this app is used on a tablet in the field for collecting phenotypic data. These data can then be uploaded into another system that the data management team is working on, called Tripal Breeding Information Management System (TripalBIMS).

TripalBIMS is a comprehensive management and analysis system for breeding data that will allow RosBREED breeders to upload and edit their data, design field plantings, determine parents for crossing, search and download data, and do statistical analyses for breeding decisions.

Behind managing all of this big data is a big team. In addition to Dorrie as the team leader, Sook Jung manages gene and trait curation and is leading the RosMAP activity. Taein Lee has worked to develop the current Breeders’ Toolbox and is developing all aspects of TripalBIMS. Ping Zheng does transcriptome and genotyping-by-sequencing (GBS) analyses as well as SNP array development, as needed by the DNA Testing Team. Chun-Huai Cheng is the web manager and also uploads genomics data and does functional analysis work. Jodi Humann helps with data curation. Heidi Hough manages computational systems and databases, while adding new data to the peach

Figure 1. The Data Management Team at WSU (Dorrie and Sook were at RGC8).
around us (my husband Jim Olmstead was a postdoc for the breeding program), Amy calmly started preparing the tree for pollination, deftly removing the existing pollen source to emasculate flowers and make her crosses. She can move through an entire tree so quickly! For those of us that are breeders or that have helped breeders make crosses, emasculating flowers successfully and consistently is tough, but Amy said that “it takes practice and a sharp edge to make the cut in the right place to remove the anthers and petals”.

A Pathway to RosBREED

The RosBREED project is a great success story of collaboration among the worldwide Rosaceae community that has taken many years. The first seed for the RosBREED program began when the Rosaceae scientific community, with industry input, began working synergistically through the establishment of the U.S. Rosaceae Genomics, Genetics, and Breeding Executive Committee (RosEXEC). An important early task of RosEXEC was to develop a visionary White Paper that described the imminent to medium-term research needs for rosaceous crops. As Chair of RosEXEC at that time, Amy mobilized the research community to ensure the White Paper was completed and recognized by the USDA as a major community accomplishment.

References:

Featured Team Member: Amy Iezzoni
Mercy Olmstead, Extension Team Leader, University of Florida

This column in the past has featured new participants to the RosBREED project, yet it’s been a while since we highlighted our project director, Dr. Amy Iezzoni. Amy has been a driving force behind the RosBREED projects and collaboration among Rosaceae scientists worldwide.

Amy Iezzoni is a professor at Michigan State University and has been with the university since 1981. Her research program covers tart cherry scion and cherry rootstock breeding. Originally she also led the peach breeding program, until the arrival of Bill Shane, currently located at the Southwest Michigan Research and Education Center in Benton Harbor, MI. Together in 2007 they released ‘Beaumont’ peach. Bill Shane is now a demonstration peach breeder in RosBREED.

Amy teaches graduate courses in the Plant Breeding, Genetics, and Biotechnology program in advanced plant breeding and a module on polyploidy. Many of her own graduate students have gone into the private sector and in academia to become successful breeders, including several students involved in the RosBREED project. Several students have stayed involved with RosBREED and gone on to advanced degrees or postdoctoral appointments with various professors.

One of my favorite memories of Amy is when she came out to the WSU Irrigated Agriculture Research and Extension Center at Prosser to help direct and make sweet cherry crosses. While the bees were buzzing around us (my husband Jim Olmstead was a postdoc for the breeding program), Amy calmly started preparing the tree for pollination, deftly removing the existing pollen source to emasculate flowers and make her crosses. She can move through an entire tree so quickly! For those of us that are breeders or that have helped breeders make crosses, emasculating flowers successfully and consistently is tough, but Amy said that “it takes practice and a sharp edge to make the cut in the right place to remove the anthers and petals”.

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Shortly afterwards, sequencing had been completed on the apple genome (Velasco et al., 2010), the diploid strawberry genome (Shulaev et al., 2011), and the peach genome (Arús et al., 2012) which provided powerful foundations for RosBREED to develop infrastructure.
and, ultimately, the DNA information that breeders can use in their program. The first project, 2009-2014, built many of the platforms that are now being used by breeders in practice and in new RosBREED 2 research to combine superior horticultural quality with disease resistance. Leveraging community resources has reduced redundancies among research groups in Rosaceae and has rapidly advanced discoveries and their translation to practice.

In addition to the multitude of infrastructure resources that RosBREED has helped to develop - student training has been a resounding success! Amy has mentored her fair share of students in Rosaceae crop breeding over the years, many of whom have gone into both academia and private industry. Certainly the impact that she has had on their lives and associated scientists in the Rosaceae community has been great.

What’s one thing people may not know about you?

“I’m a farmer – I know what it’s like to lose a crop. I know what the costs are and the forms that need to be filled out. I know how challenging it is.” Amy and her husband, Charlie Edson, are owners of Bel Lago Vineyards and Winery, located on the Leelanau Peninsula in northwestern Michigan. Amy said that operating a farm has “helped her focus with RosBREED on what we can deliver – it’s not just an academic exercise.”

References


Phinally Done

Dr. Paul Sandefur

Congratulations to Dr. Paul Sandefur, who successfully defended his dissertation at Washington State University, entitled "Enhancing efficiency in tree-fruit breeding by developing trait-predictive DNA tests".

Paul has spent six years working on research within the RosBREED project, first earning a Master’s Degree with Dr. John Clark at the University of Arkansas before joining the program of Dr. Cameron Peace.

“I’m very thankful to have had the opportunity to be a part of the RosBREED project throughout my graduate studies,” Dr. Sandefur said.

Sandefur’s post-Ph.D. plans include continuing his work with peaches, apples, and sweet cherries at WSU with Dr. Cameron Peace, and he said he hopes to continue working as a fruit breeder in academia after that.

Dr. Sushan Ru

Hats off to Dr. Sushan Ru, who earned her Ph.D. from Washington State University. Her advisers were Drs. Cameron Peace and Dorrie Main, both of whom she credits with spurring her interest in tree fruit breeding.

Ru’s dissertation topic was “Theoretical and empirical evaluation of efficiency of marker-assisted seedling selection in Rosaceae tree fruit breeding”. The goal of her research was to facilitate wider and more successful adoption of marker-assisted seedling selection (MASS) by helping out with one of the most prevalent challenges: the difficulties in estimating cost and genetic efficiency of MASS compared to traditional seedling selection.

Ru plans to spend the next year as a postdoc at WSU with Dr. Dorrie Main, after which she hopes to continue working in areas related to translational genetics and its applications in plant breeding.

Keep up to date with the latest news from RosBREED: https://www.rosbreed.org/whats-new
Angular leaf spot (ALS) is a major bacterial disease of cultivated strawberry (Fragaria × ananassa) caused by Xanthomonas fragariae (Kennedy and King, 1960 and 1962a). The pathogen colonizes strawberry leaves, thereby reducing the overall productivity and yields of affected plants. Symptoms of the disease include small, water-soaked lesions on the underside of leaves that eventually enlarge to form angular spots that are demarcated by leaf veins (Figure 1). At the beginning stages of infection, the small lesions appear translucent when viewed with transmitting light, but appear dark green when viewed without additional supplemental light. This distinction can be important in identifying the disease. Lesions can emit a bacterial ooze under conditions of high humidity and this ooze provides secondary inoculum for the spread of the pathogen. As the severity of infection increases, foliar lesions enlarge, coalesce, become visible from the surface of leaves, and the affected tissues undergo necrosis (i.e., tissue death). Damaged leaves eventually take on a tattered appearance, spreading across an entire field (Figure 2).

Angular leaf spot can become systemic in a plant and may also infect the sepals adjacent to the fruit, causing the calyx to desiccate and darken. Although direct damage to the fruit is not caused by this pathogen, manifestation of symptoms on the calyx can reduce the marketability of fruit. Angular leaf spot was first reported in Minnesota in 1960 (Kennedy and King, 1960). Although the impact of the disease is significant worldwide, it especially affects fresh strawberry production in Florida, the second largest national producer, with production in 2014 estimated at 116,000 U.S. tons of fruit from 10,900 acres [National Agriculture Statistics Services (NASS), 2015]. The disease routinely causes a total yield loss of up to 7.4 percent by weight during the winter season and 8.6 percent loss in marketable yield due to lesions on fruit calyces (Roberts et al., 1997). The annual estimated cost to the industry is $17 million during winters with multiple hard freezes.

This bacterium is spread by several methods. In subtropical production regions like Florida, primary inoculum is likely from asymptomatic transplants with latent infections (Roberts et al., 1996). Additionally, overhead irrigation for establishment of transplants and frost protection is intensively practiced in Florida, which exacerbates the spread of ALS (Figure 3). Elsewhere, ALS is spread by rainfall to new sites of infection and spread across a field. Few viable options exist for the management of ALS. Growers are advised to plant certified planting material from reputable nurseries that are free of ALS. Infected plants can be physically removed from a planting, but this control strategy is difficult to implement at a commercial scale. Similarly, avoiding the spread of secondary inoculum is challenging for Florida growers, as overhead irrigation is essential for establishment and freeze protection in this region. Chemical control options are available through copper formulations or acibenzolar-s-methyl, but these products have limited efficacy, might damage the strawberry plant through phytotoxic effects, and are seldom used (Mertely et al., 2010).

Although there are presently no viable management options to mitigate the devastation of ALS, tools that can help the strawberry industry overcome this disease exist through the use of plant genetics, and particularly, DNA-informed breeding. This is where the efforts of Vance Whitaker, his team of allied scientists, and RosBREED come into action.

One of the most powerful tools against ALS is genetic resistance to the disease. Commercial cultivars of strawberry presently do not have any resistance to ALS and only a few cultivars exhibit minor tolerance. Just this year, Whitaker and his team identified FaRXf1, a single locus identified in wild accessions US4808 and US4809 that confers resistance to four types (Cont. on Page 6)
of X. fragariae (Roach et al., 2016). The resistance allele at this locus is being introgressed into Florida germplasm via DNA-informed breeding approaches. Several preliminary DNA tests have already been developed as a result of this locus’ discovery and are being used for marker-assisted seedling selection in Dr. Whitaker’s breeding program. These DNA tests have been made available to fellow RosBREED scientist Nahla Bassil (USDA-ARS, National Clonal Germplasm Repository in Corvallis, Oregon). As a result, Bassil can test other RosBREED strawberry material that breeders submit to her, accelerating the process of breeding for ALS resistance.

Currently, a DNA test is being perfected so that strawberry breeders around the world can test for ALS resistance. All of this demonstrates the power of collaborative research and breeding, which provides an important foundation for RosBREED to develop cultivars with resistance to diseases that threaten our important rosaceous crops.

References:

Community Events

American Society for Horticultural Science (ASHS) Annual Conference
Sheraton Atlanta Hotel, Atlanta, GA
August 8 – 11, 2016
For more information: www.ashs.org/?page=GeneralConference

International Strawberry Symposium
Quebec City Convention Centre, Quebec City, Quebec, Canada
August 13-16, 2016
For more information: www.iss2016-quebec.org/en

National Association of Plant Breeders (NAPB) Annual Meeting
Hosted by Cotton Incorporated and North Carolina State University, Raleigh, NC
August 15-18, 2016
For more information: www.plantbreeding.org/annual-meeting-2016

Jewels in the Genome: Bp-2 for Bitter Pit in Apple
Amy Iezzoni, Project Director, Michigan State University

Bitter pit in apple is a fruit physiological disorder often expressed during postharvest storage whereby soft discolored “pits” develop on the outside of the fruit flesh, rendering the fruit unmarketable. Cultural practices such as calcium post-harvest dips are used to minimize the incidence of bitter pit; however, these treatments are not sufficient for particularly susceptible cultivars such as ’Honeycrisp’, and storage losses still occur.

Developing apple cultivars with the reduced susceptibility to bitter pit has long been a priority for apple breeders. An initial investigation suggested that two major loci, named Bp-1 and Bp-2, are involved in the genetic control of bitter pit susceptibility (Korban and Swiader, 1984). Two additional investigations identified and validated a large-effect bitter pit locus on apple LG16 which is believed to represent the previously described Bp-2 (Kumar et al., 2013, Buti et al., 2015).

Two SSR markers (Hi22f06 and CH05c06) most closely associated with Bp-2 were found to be predictive of bitter pit susceptibility. Severe bitter pit symptoms among 106 progeny were 100% associated with the homozygous genotype 236/236 for Hi22f06 and the genotype 115/123 for CH05c06 (Buti et al., 2015). Therefore, these SSRs can be used to determine desirable parental combinations and eliminate bitter pit-susceptible seedlings prior to field planting. However, use of this diagnostic test for bitter pit should be coupled with knowledge of the neighboring trait locus that strongly influences fruit acidity, crispness, and juiciness (Ma, https://www.rosbreed.org/sites/default/files/resources/newsletters/RosBREED_Jewels_Malocus_apple.pdf) and resides less than 5 cM from Bp-2.

Available DNA tests for Ma (Xu et al., 2012 and “Ma-indel” used at Washington State University) and Bp-2 can be used together to design crosses and select progeny that have desirable eating quality attributes with reduced bitter pit incidence. Therefore, because knowledge of this genetic region will lead to more effective breeding of apple cultivars, it is featured as a RosBREED “Jewel in the Genome.”

References
In contrast, genetic engineering (GE) can allow more rapid and precise introduction of specific alleles. There is no crossing of parents, so sets of alleles are not reshuffled. Alleles introduced by genetic engineering might be from closely related species, from distantly related or unrelated species that cannot hybridize in nature, or from synthetically constructed DNA sequences. Cultivars entering into a genetic engineering process usually are themselves the outcome of prior selective breeding efforts. Genetically engineered organisms (sometimes referred to as genetically "modified" organisms or GMOs) are the outcome of using genetic engineering techniques somewhere in their development. These organisms are left with some inserted DNA, regardless of what other techniques have also gone into preparing them for commercial success. The genetic engineering technique therefore results in an individual that has one or a few desired attributes added to the original organism such as a plant cultivar or wild salmon species.

**Selection hurdles or regulatory and market hurdles**

Selective breeding creates many unique individual offspring, each with a hodgepodge of attributes. Typically, only a few individuals out of thousands of offspring have any hope of becoming a commercial success—most have too many negative attributes or even a single fatal flaw. To find the needles in the haystacks, the centuries-old art and modern science of breeding have honed the process of selection, with ever-increasing power due to advances in genetics, physiology, pathology, and other disciplines. In selective breeding, rigorous cycles of choosing individuals with the right combinations of desirable attributes and discarding the majority of offspring (those without desired combinations of attributes) required to reach some semblance of a breeders’ goal.

With DNA-based diagnostic tools that reveal the genetic potential of breeding individuals, the selection process for U.S. Rosaceae crop breeders becomes more efficient and accurate. Using the technique of DNA profiling, breeders can peer in at the alleles themselves—rather than their effects, which are often confounded by the weather and other hard-to-control external factors. Trait-predictive DNA tests, such as the ones offered by RosBREED, cost just a few cents to a few dollars per plant and take just a few weeks to deliver results. Large-scale breeding programs include thousands of plants, and it can take several years for each seedling to grow to maturity; then they are usually evaluated for several more years. By using DNA-informed breeding techniques to eliminate seedlings carrying undesirable alleles, breeders can focus on growing the individual plants that meet their priorities without wasting years of resources on the ones that won’t. Even after they have been selected with DNA information at the seedling stage, the best seedlings still must be grown to maturity and winnowed down further in field evaluations, and perhaps by additional DNA tests as well. The advanced selections arising from this wave of seedlings then go through one or more rounds of clonal propagation—another 5 to 15 years in the case of tree fruit.

Genetic engineering approaches, which are proliferating rapidly, may be used to add specific alleles using more rapid technical means that are not constrained by the natural ability of parents to produce offspring. This technology has led to some applications in which desirable crop characteristics have been rapidly achieved. However, the potential for unintended consequences of methods that are not constrained by normal reproductive processes is of concern to society. One result of this concern is a high level of scrutiny and skepticism on the part of consumers and global regulatory agencies. This scrutiny in turn leads to regulatory and market challenges for genetically engineered crops, which are not faced by new cultivars created by selective breeding programs—including those that take advantage of DNA information to streamline and enhance their work.

**No controversy or some controversy**

Because it only monitors alleles that organisms have inherited by natural crossing, DNA-informed breeding is not controversial. Debates on the safety of genetically engineered cultivars, and whether they should be labeled for consumers, have been going on for years. Much of the discussion has not been productive because it has argued for an all-or-nothing answer: genetic engineering is either always safe and good or never safe and good. Recently, more attention has been focused on the difficulty of determining if and when a specific genetic engineering application and outcome is likely to produce more benefits than risks, both for human consumption directly and for natural and agricultural ecosystems. But in any case, as part of the centuries-old lineage of selective breeding, DNA-informed breeding is not part of this controversy.

**RosBREED is into it or it isn’t**

Both DNA-informed breeding and genetic engineering are technological approaches for developing new cultivars that provide sustained crop-based genetic solutions to societal needs. But RosBREED’s science does not produce genetically engineered cultivars. DNA-informed breeding can be an efficient, and relatively easy way to make breeders’ jobs easier. Because it relies on monitoring rather than modifying a plant’s DNA, there’s no debate about safety, regulation, or labeling. The RosBREED project has embraced the use of DNA information in selective breeding to further horticultural quality and disease resistance in Rosaceae crops, and this approach is turning out great successes thus far! For more information on our success stories, visit https://www.rosbreed.org/SuccessStories.
Community Breeders’ Page: Functional Genotype Lists – a New DNA Information article

Julia Piaskowski, DNA-Informed Breeding Team member, WSU and Cameron Peace, DNA-Informed Breeding Team Leader, WSU

Functional genotype lists are an important part of the RosBREED project to develop DNA tests. This knowledge resource consists of tables of DNA test outcomes and their predicted phenotypes for tens to hundreds of publicly available individuals (cultivars, ancestors, and germplasm collection accessions) targeted by RosBREED (Figure 1). These lists were first provided in the first RosBREED project with some of the earliest DNA tests compiled for peach, apple, and cherry (apple example: ACS1 and ACO1). A sampling of these functional genotype lists is also included in each DNA test card (see March 2016 Community Breeders Page article: https://www.rosbreed.org/node/912).

Functional genotype lists help breeders in several ways. The DNA information they contain are the basis for marker-assisted parent selection. Once the genotypes and predicted phenotypes of a parental pool is known, this information can be used to great benefit: making crosses to generate novel and promising allele combinations, stacking useful alleles, and greater understanding of the allelic diversity of a breeding program’s germplasm. If a breeding program uses any of the germplasm included on these lists, this resource has direct applicability. If a program’s germplasm is related to individuals in the RosBREED functional genotype lists, knowing the functional genotypes of related germplasm can help breeders understand their own material better. Nevertheless, we recommend breeders test new material before making any conclusions regarding the allelic state of any particular individual at a locus.

We recommend that your program begin crafting functional genotype lists for your own germplasm. We intend the RosBREED lists to be a helpful launching point for Rosaceae breeders. We recognize that breeders are likely to use a broader array of germplasm for crossing, including proprietary germplasm unique to each program. A good starting point is to obtain functional genotypes for parents used frequently in crosses. As always, a DNA test must be validated in a breeding program by comparing predicted phenotypes with historical records of actual performance for some representative individuals. Continuing to update a functional genotype list as new parents are incorporated into a breeding program is recommended.

At this time, RosBREED has uploaded functional genotype lists for all the peach, cherry, apple, and strawberry DNA tests available, cherry fruit size, cherry self-incompatibility, and apple storability. In the next six months, look for functional genotypes from the apple DNA tests of fruit acidity, bitter pit susceptibility, fruit sweetness/fructose content, scab incidence, fruit flesh color, fruit crispness, and fruit firmness, and from cherry DNA tests of fruit firmness, fruit flesh color and skin color, powdery mildew response, and harvest timing.

Figure 1: Functional genotype list example of a few cultivars for the peach DNA test of Ppe-Acid, which predicts genetic potential for acidity based on two unlinked trait loci. The graph shows some possible genotypic outcomes and the acidity phenotype predicted to be associated with each.
Coming up in the next issue:

- Our “Meet our AP Members” column returns!
- Scenes from the Rosaceae Genomics Conference in Angers, France
- What does the DNA Testing Team do? Find out in the next issue!
- What will be the next Rosaceae Nemesis? It might be one important for your Rosaceae crop!

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